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## Figure 1A
**Binding of the mab A76-A/C7 (type GD-1) to a MUC1 30-mer depending on the glycosylation to the PDTR motif**
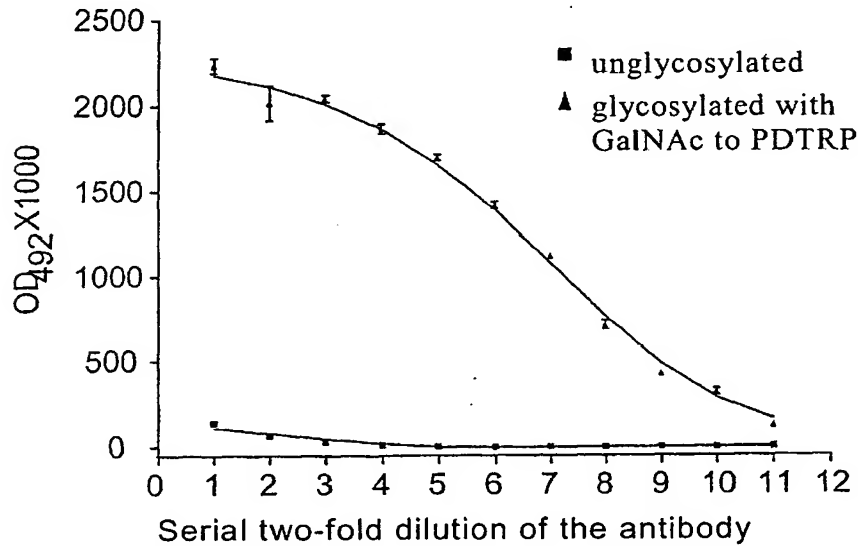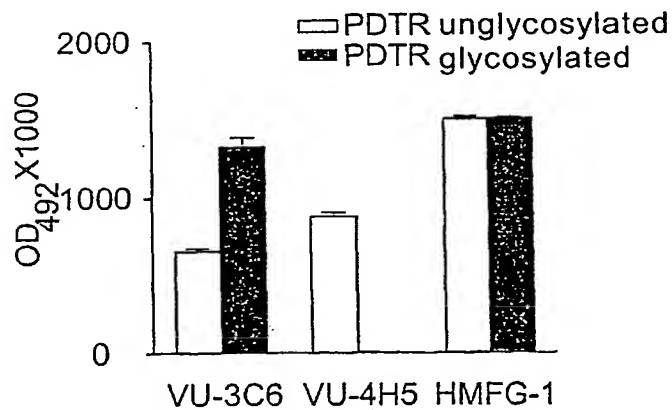


- ■ unglycosylated
- ▲ glycosylated with GalNAc to PDTRP

Y-axis: $OD_{492} \times 1000$
X-axis: Serial two-fold dilution of the antibody

## Figure 1B
**Binding pattern of mab against MUC1 of the epitope with GalNAc: VU-3C6 (type GD-2), VU-4H5 (type iGD), HMFG-1 (type GI)**
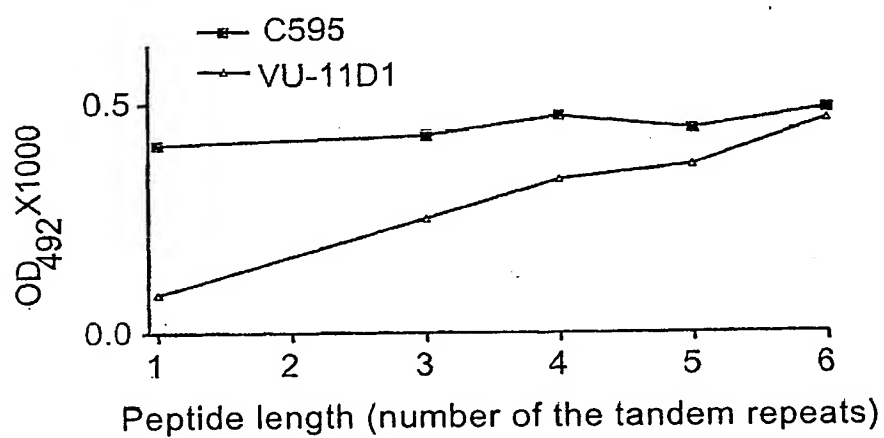


- ▢ PDTR unglycosylated
- ▨ PDTR glycosylated

Y-axis: $OD_{492} \times 1000$
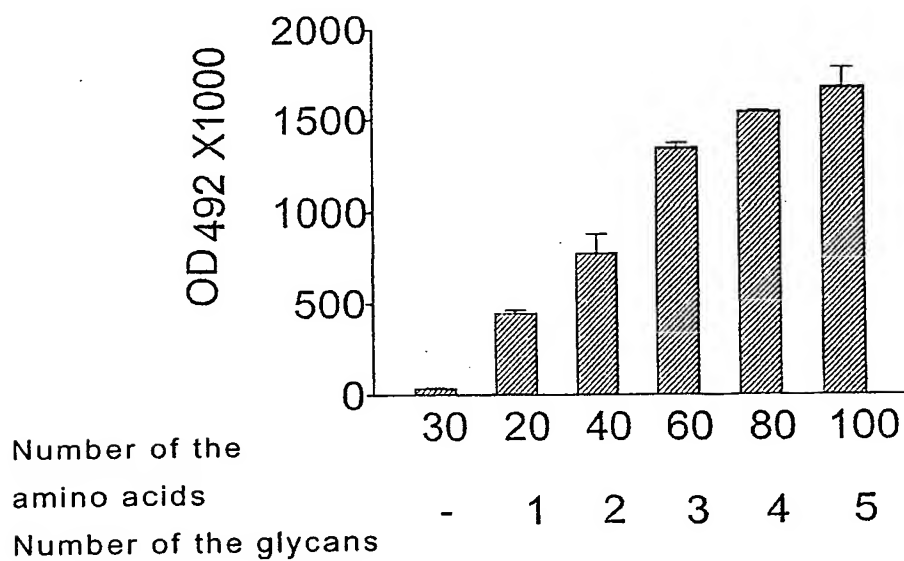X-axis: VU-3C6   VU-4H5   HMFG-1

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## Figure 2



Figure 2

# Figure 3A

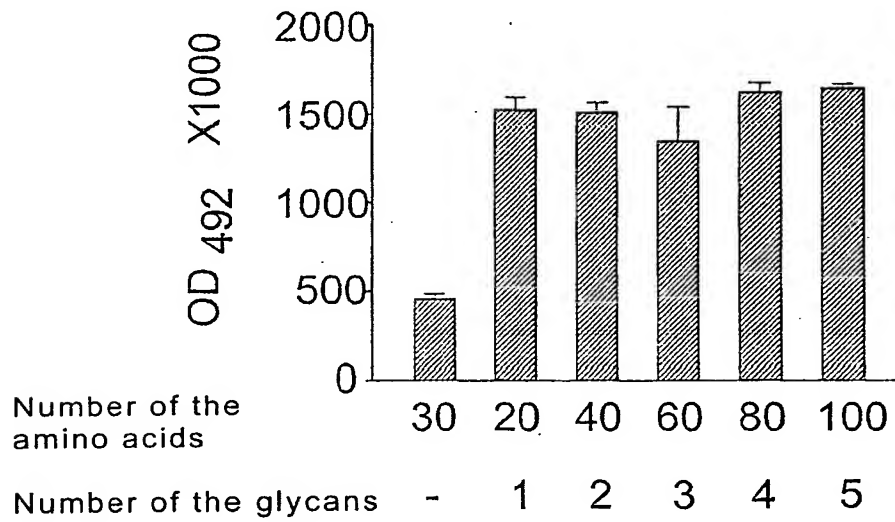Binding of the mab A76-A/C7 to glycosylated MUC1 peptides of different length (1-5 tandem repeats)
(glycosylated with Tn to PDTR motif)



Number of the amino acids

Number of the glycans

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# Figure 3B

Binding of the mab Mc5 to glycosylated MUC1 peptides of different length
(1-5 tandem repeats)
(glycosylated with Tn to PDTR motif)
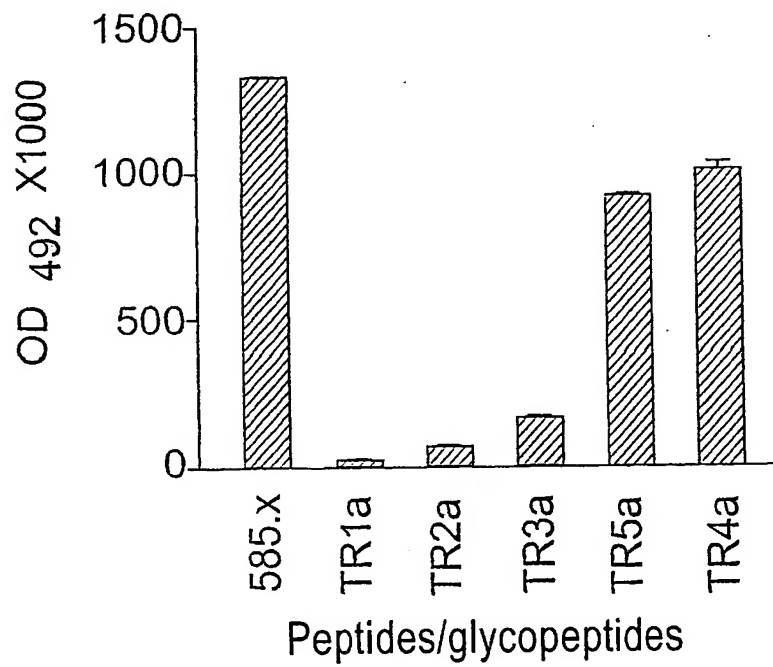
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# Figure 4

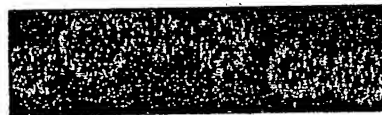| Binding of the mab VU-4H5 to glycosylated MUC1 peptides of different length (1-5 tandem repeats) (glycosylated with Tn to PDTR motif) |
| --- |

# Figure 5A

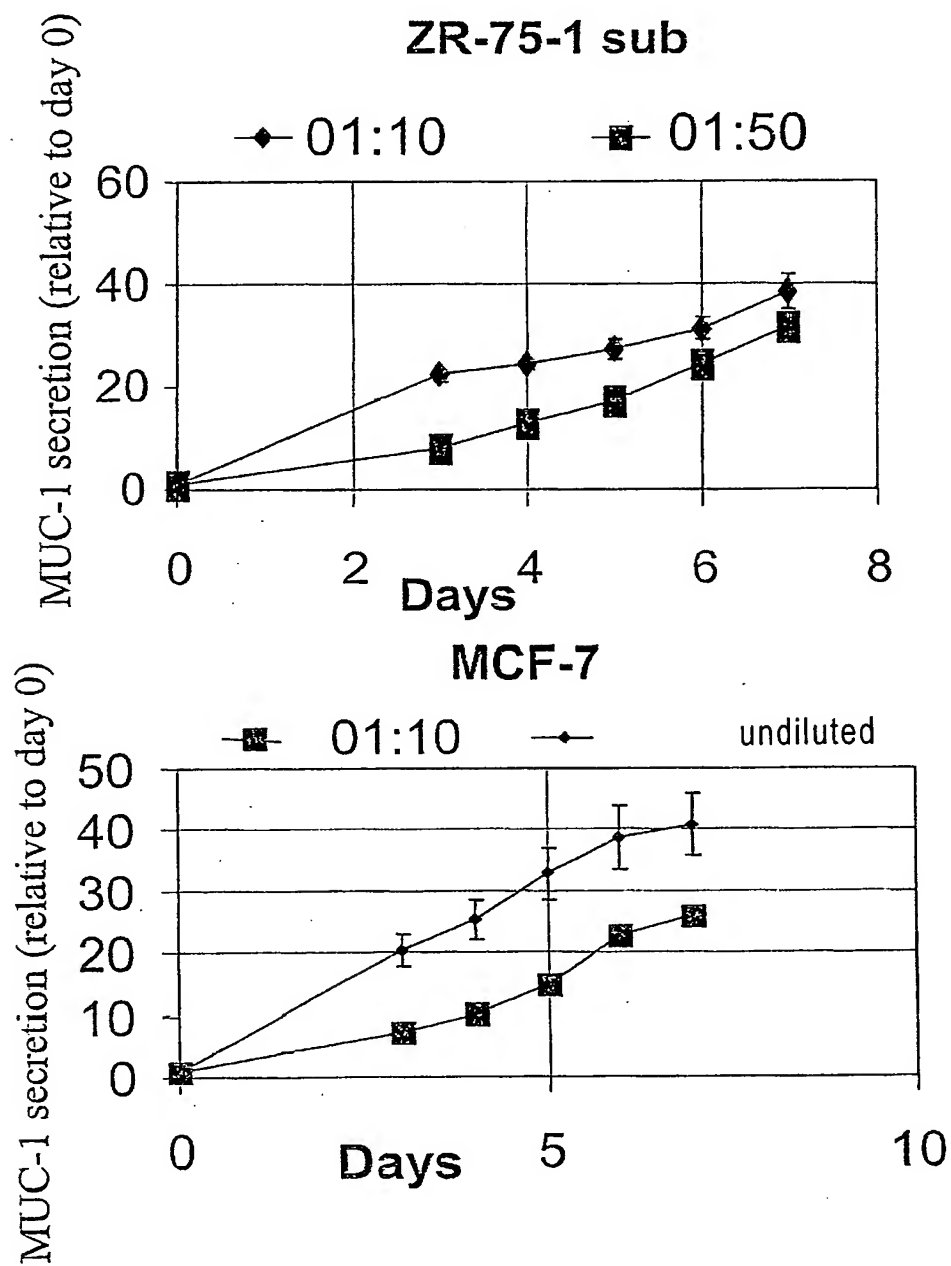1) ZR-75-1 cells before accumulation with A76-A/C7 and cloning

2) ZR-75-1 cells after accumulation with A76-A/C7 and cloning (ZR-75-1-sub)
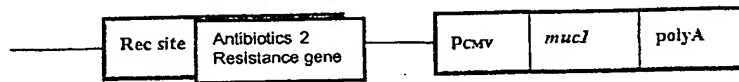
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**Figure 5 B**



ZR-75-1 sub

MCF-7

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## Figure 5C (1)

A. Expression vector

| Rec site | Antibiotics 2 Resistance gene | | $P_{CMV}$ | muc1 | polyA |

B. Sequenz of the human muc1 cDNA, which were cloned into the expression vector. The primers which were used for the amplification of the cDNA from ZR-75-1 cDNA are marked in green.

```
      GAATTCCCTG GCTGCTTGAA TCTGTTCTGC CCCCTCCCCA CCCATTTTCAG
1     ---------- ---------- ---------- ---------- ----------
      CTTAAGGGAC CGACGAACTT AGACAAGACG GGGGAGGGGT GGGTAAAGTG
              +10        +20        +30        +40
      CAGCACCATGACACGGGGCA CCCAGTCTCC TTTCTTCCTG CTGCTGCTCC
51    -------*** ********** ********** ********** **********
      GTGGTGGTAC TGTGCCCGT GGGTCAGAGG AAAGAAGGAC GACGACGAGG
              +10        +20        +30        +40
      TCACAGTGCT TACAGTTGTT ACAGGTTCTG GTCATGCAAG CTCTACCCCA
101   ********** ********** ********** ********** **********
      AGTGTCACGA ATGTCAACAA TGTCCAAGAC CAGTACGTTC GAGATGGGGT
              +10        +20        +30        +40
      GGTGGAGAAA AGGAGACTTC GGCTACCCAG AGAAGTTCAG TGCCCAGCTC
151   ********** ********** ********** ********** **********
      CCACCTCTTT TCCTCTGAAG CCGATGGGTC TCTTCAAGTC ACGGGTCGAG
              +10        +20        +30        +40
      TACTGAGAAG AATGCTGTGA GTATGACCAG CAGCGTACTC TCCAGCCACA
201   ********** ********** ********** ********** **********
      ATGACTCTTC TTACGACACT CATACTGGTC GTCGCATGAG AGGTCGGTGT
              +10        +20        +30        +40
      GCCCCGGTTC AGGCTCCTCC ACCACTCAGG GACAGGATGT CACTCTGGCC
251   ********** ********** ********** ********** **********
      CGGGGCCAAG TCCGAGGAGG TGGTGAGTCC CTGTCCTACA GTGAGACCGG
              +10        +20        +30        +40
      CCGGCCACGG AACCAGCTTC AGGTTCAGCT GCCACCTGGG GACAGGATGT
301   ********** ********** ********** ********** **********
      GGCCGGTGCC TTGGTCGAAG TCCAAGTCGA CGGTGGACCC CTGTCCTACA
              +10        +20        +30        +40
      CACCTCGGTC CCAGTCACCA GGCCAGCCCT GGGCTCCACC ACCCCGCCAG
351   ********** ********** ********** ********** **********
      GTGGAGCCAG GGTCAGTGGT CCGGTCGGGA CCCGAGGTGG TGGGGCGGTC
              +10        +20        +30        +40
      CCCACGATGT CACCTCAGCC CCGGACAACA AGCCAGCCCC GGGCTCCACC
401   ********** ********** ********** ********** **********
      GGGTGCTACA GTGGAGTCGG GGCCTGTTGT TCGGTCGGGG CCCGAGGTGG
              +10        +20        +30        +40
      GCCCCCCCAG CCCACGGTGT CACCTCGGCC CCGGACACCA GGCCGCCCCC
451   ********** ********** ********** ********** **********
      CGGGGGGGTC GGGTGCCACA GTGGAGCCGG GGCCTGTGGT CCGGCGGGGG
              +10        +20        +30        +40
      GGGCTCCACC GCCCCCCCAG CCCACGGTGT CACCTCGGCC CCGGACACCA
501   ********** ********** ********** ********** **********
      CCCGAGGTGG CGGGGGGGTC GGGTGCCACA GTGGAGCCGG GGCCTGTGGT
              +10        +20        +30        +40
      GGCCGCCCCC GGGCTCCACC GCGCCCGCAG CCCACGGTGT CACCTCGGCC
551   ********** ********** ********** ********** **********
      CCGGCGGGGG CCCGAGGTGG CGCGGGCGTC GGGTGCCACA GTGGAGCCGG
```

## Figure 5C (2)

```
            +10       +20       +30       +40
     CCGGACACCA GGCCGGCCCC GGGCTCCACC GCCCCCCCAG CCCATGGTGT
601  ********** ********** ********** ********** **********
     GGCCTGTGGT CCGGCCGGGG CCCGAGGTGG CGGGGGGGTC GGGTACCACA
            +10       +20       +30       +40

     CACCTCGGCC CCGGACAACA GGCCCGCCTT GGCGTCCACC GCCCCTCCAG
651  ********** ********** ********** ********** **********
     GTGGAGCCGG GGCCTGTTGT CCGGGCGGAA CCGCAGGTGG CGGGGAGGTC
            +10       +20       +30       +40

     TCCACAATGT CACCTCGGCC TCAGGCTCTG CATCAGGCTC AGCTTCTACT
701  ********** ********** ********** ********** **********
     AGGTGTTACA GTGGAGCCGG AGTCCGAGAC GTAGTCCGAG TCGAAGATGA
            +10       +20       +30       +40

     CTGGTGCACA ACGGCACCTC TGCCAGGGCT ACCACAACCC CAGCCAGCAA
751  ********** ********** ********** ********** **********
     GACCACGTGT TGCCGTGGAG ACGGTCCCGA TGGTGTTGGG GTCGGTCGTT
            +10       +20       +30       +40

     GAGCACTCCA TTCTCAATTC CCAGCCACCA CTCTGATACT CCTACCACCC
801  ********** ********** ********** ********** **********
     CTCGTGAGGT AAGAGTTAAG GGTCGGTGGT GAGACTATGA GGATGGTGGG
            +10       +20       +30       +40

     TTGCCAGCCA TAGCACCAAG ACTGATGCCA GTAGCACTCA CCATAGCACG
851  ********** ********** ********** ********** **********
     AACGGTCGGT ATCGTGGTTC TGACTACGGT CATCGTGAGT GGTATCGTGC
            +10       +20       +30       +40

     GTACCTCCTC TCACCTCCTC CAATCACAGC ACTTCTCCCC AGTTGTCTAC
901  ********** ********** ********** ********** **********
     CATGGAGGAG AGTGGAGGAG GTTAGTGTCG TGAAGAGGGG TCAACAGATG
            +10       +20       +30       +40

     TGGGGTCTCT TTCTTTTTCC TGTCTTTTCA CATTTCAAAC CTCCAGTTTA
951  ********** ********** ********** ********** **********
     ACCCCAGAGA AAGAAAAAGG ACAGAAAAGT GTAAAGTTTG GAGGTCAAAT
            +10       +20       +30       +40

     ATTCCTCTCT GGAAGATCCC AGCACCGACT ACTACCAAGA GCTGCAGAGA
1001 ********** ********** ********** ********** **********
     TAAGGAGAGA CCTTCTAGGG TCGTGGCTGA TGATGGTTCT CGACGTCTCT
            +10       +20       +30       +40

     GACATTTCTG AAATGTTTTT GCAGATTTAT AAACAAGGGG GTTTTCTGGG
1051 ********** ********** ********** ********** **********
     CTGTAAAGAC TTTACAAAAA CGTCTAAATA TTTGTTCCCC CAAAAGACCC
            +10       +20       +30       +40

     CCTCTCCAAT ATTAAGTTCA GGCCAGGATC TGTGGTGGTA CAATTGACTC
1101 ********** ********** ********** ********** **********
     GGAGAGGTTA TAATTCAAGT CCGGTCCTAG ACACCACCAT GTTAACTGAG
            +10       +20       +30       +40

     TGGCCTTCCG AGAAGGTACC ATCAATGTCC ACGACGTGGA GACACAGTTC
1151 ********** ********** ********** ********** **********
     ACCGGAAGGC TCTTCCATGG TAGTTACAGG TGCTGCACCT CTGTGTCAAG
            +10       +20       +30       +40

     AATCAGTATA AAACGGAAGC AGCCTCTCGA TATAACCTGA CGATCTCAGA
1201 ********** ********** ********** ********** **********
     TTAGTCATAT TTTGCCTTCG TCGGAGAGCT ATATTGGACT GCTAGAGTCT
            +10       +20       +30       +40

     CGTCAGCGTG AGTGATGTGC CATTTCCTTT CTCTGCCCAG TCTGGGGCTG
1251 ********** ********** ********** ********** **********
     GCAGTCGCAC TCACTACACG GTAAAGGAAA GAGACGGGTC AGACCCCGAC
```

## Figure 5C (3)

```
            +10        +20        +30        +40
      GGGTGCCAGG CTGGGGCATC GCGCTGCTGG TGCTGGTCTG TGTTCTGGTT
1301 ********** ********** ********** ********** **********
    . CCCACGGTCC GACCCCGTAG CGCGACGACC ACGACCAGAC ACAAGACCAA
            +10        +20        +30        +40
      GCGCTGGCCA TTGTCTATCT CATTGCCTTG GCTGTCTGTC AGTGCCGCCG
1351 ********** ********** ********** ********** **********
      CGCGACCGGT AACAGATAGA GTAACGGAAC CGACAGACAG TCACGGCGGC
            +10        +20        +30        +40
      AAAGAACTAC GGGCAGCTGG ACATCTTTCC AGCCCGGGAT ACCTACCATC
1401 ********** ********** ********** ********** **********
      TTTCTTGATG CCCGTCGACC TGTAGAAAGG TCGGGCCCTA TGGATGGTAG
            +10        +20        +30        +40
      CTATGAGCGA GTACCCCACC TACCACACCC ATGGGCGCTA TGTGCCCCCT
1451 ********** ********** ********** ********** **********
      GATACTCGCT CATGGGGTGG ATGGTGTGGG TACCCGCGAT ACACGGGGGA
            +10        +20        +30        +40
      AGCAGTACCG ATCGTAGCCC CTATGAGAAG GTTTCTGCAG GTAATGGTGG
1501 ********** ********** ********** ********** **********
      TCGTCATGGC TAGCATCGGG GATACTCTTC CAAAGACGTC CATTACCACC
            +10        +20        +30        +40
      CAGCAGCCTC TCTTACACAA ACCCAGCAGT GGCAGCCACT TCTGCCAACT
1551 ********** ********** ********** ********** **********
      GTCGTCGGAG AGAATGTGTT TGGGTCGTCA CCGTCGGTGA AGACGGTTGA
            +10        +20        +30        +40
      TGTAGGGGCA CGTCGCCCTC TGAGCTGAGT GGCCAGCCAG TGCCATTCCA
1601 *****----- ---------- ---------- ---------- ----------
      ACATCCCGT GCAGCGGGAGTACTCGACTCA GCGGTCGGTC ACGGTAAGGT
            +10        +20        +30        +40
      CTCCACTCAG GGCTCTCTGG GCCAGTCCTC CTGGGAGCCC CCACCACAAC
1651 ---------- ---------- ---------- ---------- ----------
      GAGGTGAGTC CCGAGAGACC CGGTCAGGAG GACCCTCGGG GGTGGTGTTG
            +10        +20        +30        +40
      ACTTCCCAGG CATGGAATTC C
1701 ---------- ---------- -
      TGAAGGGTCC GTACCTTAAG G
```

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# Figure 5D (1)

## A.  Rec site vector

| $P_{SV40}$ | ATG | *rec site* | *lacZ-antibiotics1 resistance gene* |
|---|---|---|---|
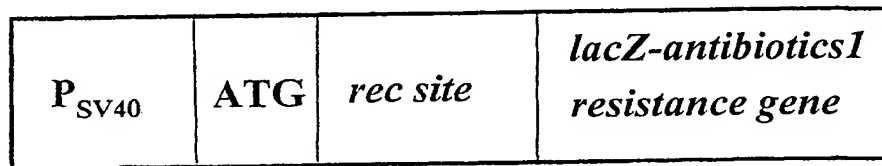
## Features of the stably transfected host cells:

- Integration of the recombination site in connection with the lacZ antibiotic1 resistance fusion gene
- Resistance against the antibiotic1, expression of the lacZ gene (β-galactosidase activity)
- The transfectants differ with respect to the strength of the expression of the fusion gene (chromosomal positioning effect) depending on the integration site of the recombination site and of the fusion gene in the chromosome. Detection by ß-galactosidase activities differing in strength.

# Figure D (2).

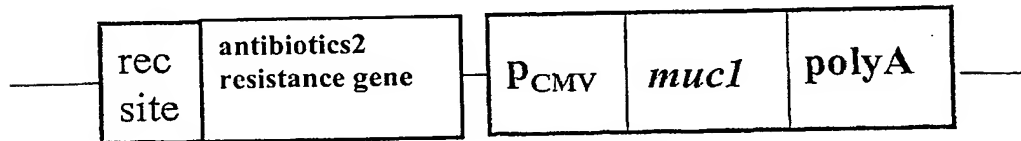A. <u>Integration of the muc1 cDNA via the rec site into the ZR-75-1 genome</u>
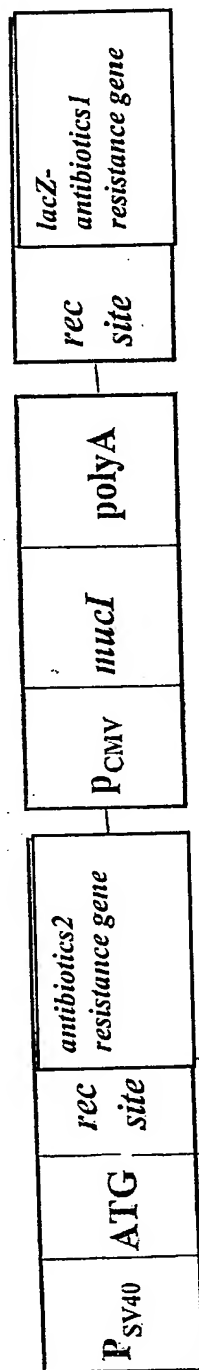
Recombinase
Expression vector

| P<sub>CMV</sub> | *Recombinase Gen* | polyA |
|---|---|---|

+

Muc1 expression vector with recombination site (rec site)

| rec site | antibiotics2 resistance gene | P<sub>CMV</sub> | *muc1* | polyA |
|---|---|---|---|---|

Product of the
side-specific
recombination

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## Figure D (3)

| P_{SV40} | ATG | rec site | antibiotics2 resistance gene |
|---|---|---|---|

| p_{CMV} | mucI | polyA |
|---|---|---|

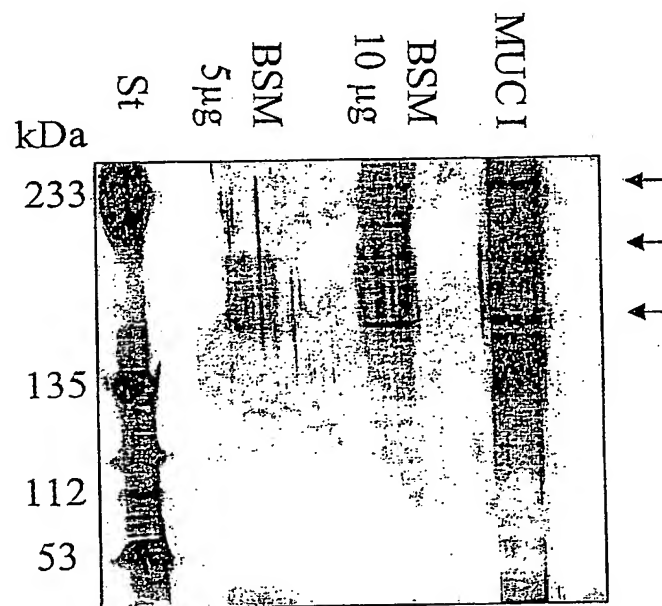| rec site | lacZ- antibiotics1 resistance gene |
|---|---|

## Features of the stably transfected host cells:

- Resistance against antibiotic 2
- Sensitivity to antibiotic 1, ß-galactosidase inactive
- High expression of the recombinant muc1 gene due to the strong promotor and the chromosomal positioning effect
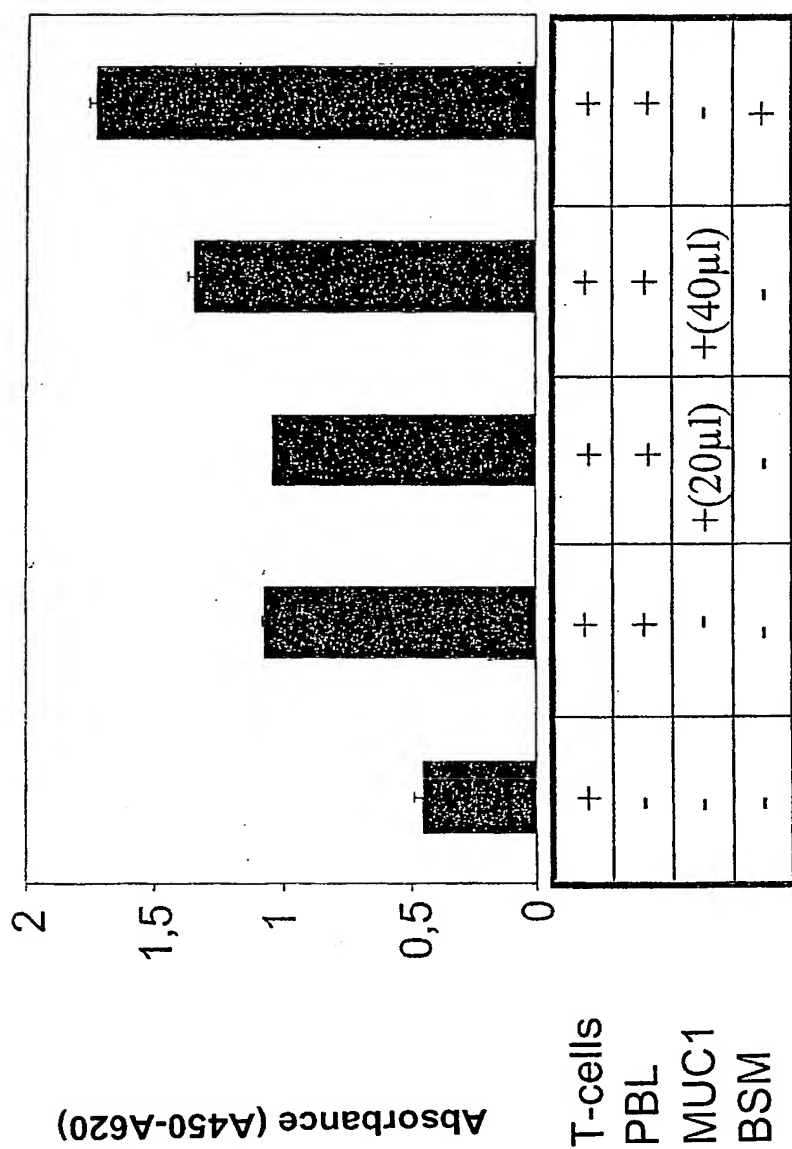
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# Figure 5E

BSM - bovine submaxillary mucin 1
MUC1 - Mucin 1 from cell supernatants

Figure 5F

| T-cells | + | + | + | + | + |
|---------|---|---|---|---|---|
| PBL | - | + | + | + | + |
| MUC1 | - | - | +(20µl) | +(40µl) | - |
| BSM | - | - | - | - | + |

Absorbance (A450-A620)

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## Figure 6



Target cell:
- ☐ MCF7
- ▨ LS174T
- ■ T2 + MUC1
- ▨ T2 + HIV

Specific lysis [%]

Ratio (target cell : effector cell)
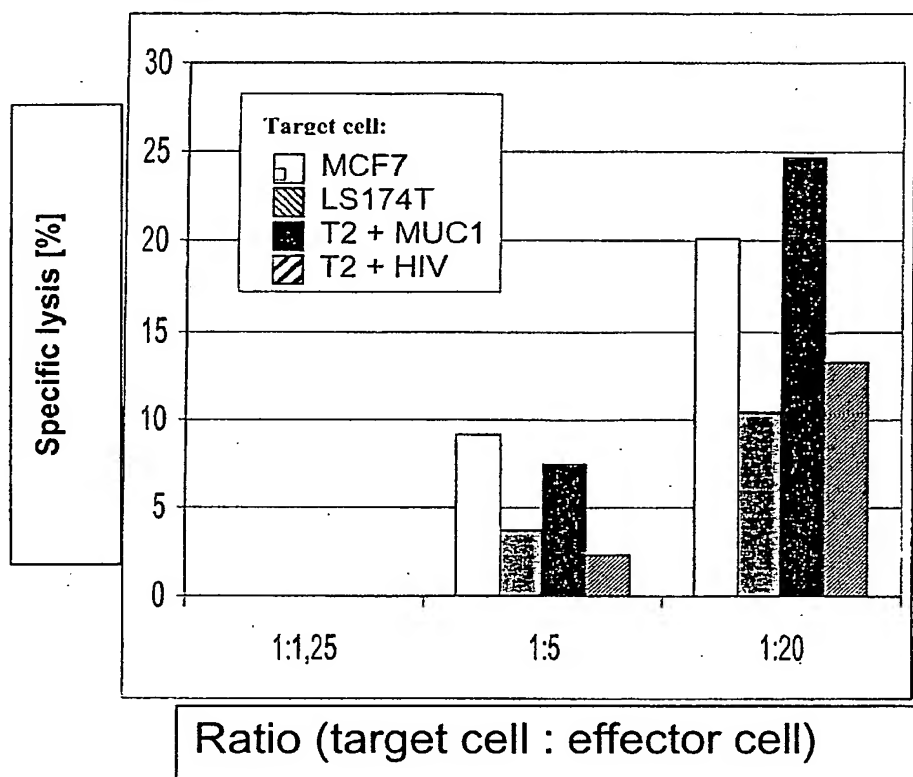
|         | % SR |
|---------|------|
| MCF7    | 8,86 |
| LS147T  | 7,58 |
| T2 + MUC1 | 3,18 |
| T2 +HIV | 4,37 |